

# Effects of Toxaphene on Hepatic Enzyme Induction and Circulating Steroid Levels in the Rat

by David B. Peakall\*†

Rats were given a single dose of toxaphene (120 mg/kg, equivalent to 1/2 LD<sub>50</sub>) and sacrificed at 1, 5, and 15 days. Liver weight and hepatic microsomal enzyme activity were increased at day 5 and 15. The level of plasma testosterone was significantly decreased at day 15. In a second experiment rats were given 2.4 mg/kg daily and sacrificed at 1, 3 and 6 months. Liver weights and microsomal enzyme activity were significantly increased over controls; enzyme activity was, however, decreasing by the end of the experiment. Plasma testosterone levels were not affected. It is concluded that enhanced hepatic enzyme induction causes only a transient drop in circulating testosterone levels followed by a return to normal values.

## Introduction

The discovery by Hart, Shultice, and Fouts (1) of hepatic enzyme induction by organochlorines opened a large and fruitful area of research. In 1967, Conney reviewed 379 papers in 49 pages in a review (2) entitled "Pharmacological implications of microsomal enzyme induction." Any comprehensive review today would require a book. Hepatic enzyme induction is caused by a wide variety of compounds and has been demonstrated to cause enhanced metabolism of an even wider range of substrates (3). The structure-activity relationship of this induction of enzyme activity has been considered, but no clear-cut patterns have been observed (2, 4). Microsomal enzyme induction by organochlorines has been demonstrated in a wide variety of species, from primates (5) to flies (6).

My own particular interest is in the physiological importance of these changes to the intact

animal rather than in the details of the mechanism. In this regard, the finding that liver microsomal enzymes metabolize not only drugs and other foreign substances, but also a variety of normally occurring compounds, is of interest. One of the most important classes of compounds metabolized are the steroid hormones. Although a good many studies have been made on the *in vitro* metabolism of steroids by microsomal enzymes, studies on the effect in the intact animal have been largely lacking. The basic question to be answered is whether chronic exposure to organochlorines causing hepatic enzyme induction has any effect on circulating steroid levels. In one short-term experiment on doves (7) a decreased level of estradiol associated with increased hepatic enzyme levels was demonstrated. However, it is possible that in long-term experiments the feedback mechanisms that normally operate to regulate hormone production may compensate for this increased steroid metabolism and thus cause no final alteration of hormone levels. Toxaphene was chosen for this study as this material is still in use in both the U.S. and U.S.S.R., whereas such widely studied materials as DDT and dieldrin are largely banned from use.

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## Materials and Methods

The experiments were divided into two sections, short-term and chronic administration. In the short-term experiments male rats were given toxaphene orally by capsule, a single dose of 1/2 LD<sub>50</sub> (120 mg/kg), and the animals sacrificed at 1, 5, and 15 days. In the chronic experiments the animals were fed 1/100 LD<sub>50</sub> daily for 1, 3, and 6 months. At sacrifice blood samples were taken and the liver removed and weighed. The liver was homogenized in 0.25M sucrose (1:7) using a Teflon/glass homogenizer maintained in ice water. Cell debris and mitochondria were removed by centrifugation at 18,000g at 4°C. The assay for enzyme activity was essentially that of Conney and Klutch (8), except that an aliquot of the supernatant from the 18,000g centrifugation was used rather than resuspended microsomal fraction. Testosterone-4-C<sup>14</sup> was used as a labeled substrate. At the end of the incubation the steroids were extracted with dichloromethane and separation of the polar metabolites carried out by paper chromatography. A typical separation is shown in Figure 1.

Assays of plasma testosterone were carried out by radioimmunoassay. The commercially available kits from New England Nuclear were used. In this procedure approximately 1000cpm of labeled steroid was added to the plasma before extraction so that the percentage recovery could be calculated. Extraction was carried out with diethyl ether, followed by separation of the steroids by column chromatography on Sephadex LH-20. Part of the sample was used to calculate recovery and part for radioimmunoassay. For the assay procedure a portion of the steroid solution was evaporated to dryness and redissolved in phosphate buffer. Labeled steroid was added and also antiserum, and after

mixing the tubes were maintained at 4°C for 4 hr. Then a suspension of dextran-coated charcoal was added, mixed, and allowed to stand for 5 min. The tubes were then centrifuged at 2000g, and the supernatant was decanted into scintillation counting vials. The percentage of steroid bound to the antiserum was then calculated and the amount determined in relation to a calibration curve of standard steroid concentrations run at the same time.

## Results

The basic results are given in Table 1 and Figures 2 and 3. Toxaphene was found to increase liver weight, this increase becoming significant by

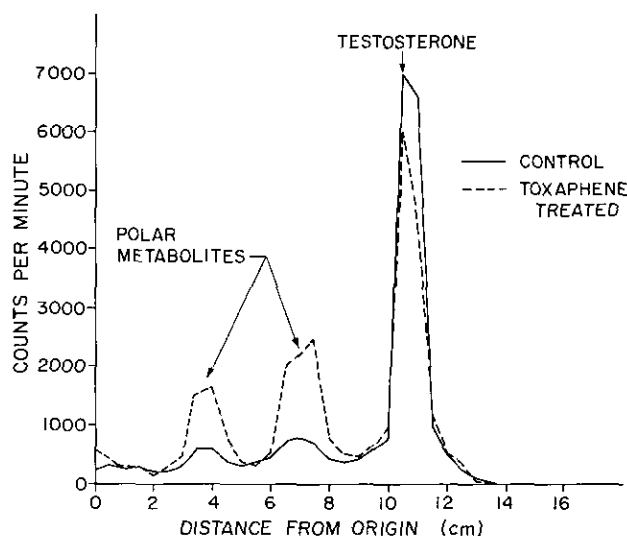


FIGURE 1. Separation of polar metabolites from unchanged testosterone by paper chromatography.

Table 1. Alterations of liver weight, microsomal enzyme activity, and circulating testosterone levels in the rat exposed to toxaphene.

Time on dose, (days)	No. of animals	Liver weight, g-%/body weight <sup>a</sup>			Microsomal enzyme activity, $\mu$ mole substrate/g liver <sup>a</sup>				Plasma testosterone level, ng/100 ml <sup>a</sup>		
		Mean	S.D.	Signf.	Mean	S.D.	Signf. (control)	Signf. (15 days)	Mean	S.D.	Signf.
0	10	4.08 $\pm$ 0.20		—	343 $\pm$ 47		—	0.001	533 $\pm$ 54		—
1	5	4.14 $\pm$ 0.21		N.s.	345 $\pm$ 45		N.s.	0.001	525 $\pm$ 43		N.s.
5	5	4.43 $\pm$ 0.15		0.02	1660 $\pm$ 124		0.001	0.050	430 $\pm$ 84		0.02
15	5	4.68 $\pm$ 0.11		0.01	1824 $\pm$ 97		0.001	—	524 $\pm$ 63		N.s.
30	5	4.84 $\pm$ 0.14		0.01	1735 $\pm$ 78		0.001	N.s.	546 $\pm$ 48		N.s.
90	5	4.85 $\pm$ 0.14		0.01	1667 $\pm$ 82		0.001	0.020	538 $\pm$ 51		N.s.
180	5	4.77 $\pm$ 0.20		0.01	1585 $\pm$ 106		0.001	0.010	535 $\pm$ 32		N.s.

<sup>a</sup>N.s. = not significant; S.D. = standard deviation.

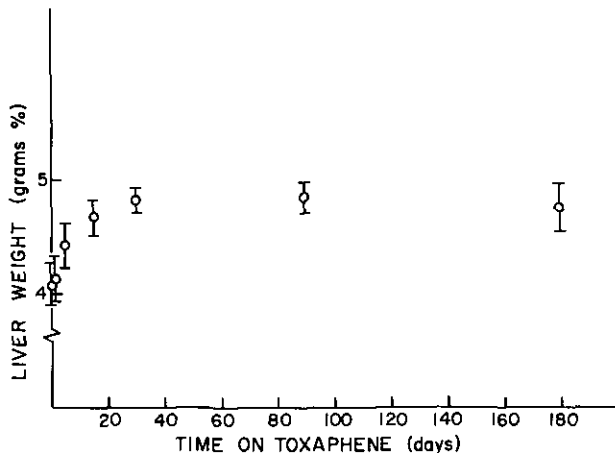


FIGURE 2. Variation of liver weight with time on toxaphene diet.

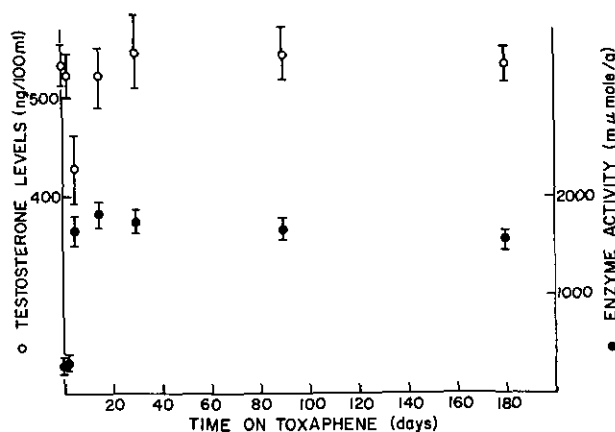


FIGURE 3. Variation of plasma testosterone levels and hepatic enzyme activity with time on toxaphene diet.

day 5. Hepatic enzyme induction was marked, a significant increase again occurring by day 5. There is a distinct tendency for enzyme activity to fall with prolonged exposure, and values obtained at 3 and 6 months are significantly lower than that obtained at 15 days. Testosterone levels were lower at day 5 ( $p = 0.02$ ) but had returned to control values by day 15 and did not significantly change thereafter.

## Discussion

The work on the testosterone levels needs to be extended to cover the first 10 days or so after exposure in more detail. The indications from the current studies are that greatly enhanced hepatic metabolism of testosterone leads to a transient

drop in the circulating testosterone level, but that this is rapidly compensated for by increased production. These findings are in agreement with those of Wedig and Gay (9), who found that phenobarbital and chlordane both increased hepatic microsomal hydroxylating activity but failed to prevent luteinizing hormone release or ovulation in the rat. Working along similar lines, Orberg and Lundberg (10) found that DDT and PCBs were able to reduce the weight of testes and seminal vesicles of castrated rats but not of intact animals. In both castrated and intact animals enhanced hepatic enzyme induction was found, and the authors concluded that the intact animal was capable of compensating for enhanced steroid breakdown, although no direct measurements of steroid levels were made. The studies reported here measure blood testosterone directly and show that the levels are only temporarily affected.

## Conclusion

The level of toxaphene used has a marked effect on the level of activity of microsomal enzymes in the liver. However, testosterone levels show only a transient drop followed by a return to normal values.

## REFERENCES

- Hart, L. G., Shultice, R. W., and Fouts, J. R. Stimulatory effects of chlordane on hepatic microsomal drug metabolism in the rat. *Toxicol. Appl. Pharmacol.* 5: 371 (1963).
- Conney, A. H. Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* 19: 317 (1967).
- Conney, A. H., and Burns, J. J. Metabolic interactions among environmental chemicals and drugs. *Science* 178: 576 (1972).
- Peters, M. A. Relative selectivity of some microsomal drug metabolizing enzyme inducers. *Arch. Int. Pharmacodyn. Ther.* 203: 30 (1973).
- Cram, R. L., Juchau, M. R., and Fouts, J. R. Stimulation by chlordane of hepatic drug metabolism in the squirrel monkey. *J. Lab. Clin. Med.* 66: 906 (1965).
- Rhee, K. S., and Plapp, F. W., Jr. Polychlorinated biphenyls (PCBs) as inducers of microsomal enzyme activity in the housefly. *Arch. Environ. Contamin. Toxicol.* 1: 182 (1973).
- Peakall, D. B. *p,p'*-DDT: effect on calcium metabolism and concentration of estradiol in the blood. *Science* 168: 592 (1970).
- Conney, A. H., and Klutch, A. Increased activity of androgen hydroxylases in liver microsomes of rats pretreated with phenobarbital and other drugs. *J. Biol. Chem.* 238: 1611 (1973).

9. Wedig, J. H., and Gay, V. L. Can increased hepatic estrogen metabolism interfere with ovulation in the rat? Effects of chronic phenobarbital or chlordane treatment. *Proc. Soc. Expt. Biol. Med.* 144: 796 (1973).
10. Orberg, J., and Lundberg, C. Some effects of DDT and PCB on the hormonal system in the male mouse. *Environ. Physiol. Biochem.* 4: 116 (1974).